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STRUCTURAL STUDY ON THE EFFECT OF TRIGONELLA FOENUM GRAECUM SEEDS ON RATS' KIDNEYS

George C. PRIBAC [1], Aurel ARDELEAN [1], Constantin CRACIUN [2], Constantin PUICA [2], Corina ROSIORU [2], Aurelia COVACI [4], Cecilia AVRAM [1], Simona DAMIAN [1], Endre MATHE [3], Maria CZAPAR [3], Lucian PAIUSAN [1], Liana MOS [1], Coralia COTORACI [1]

[1] "Vasile Goldis" Western University, Faculty of Medicine, Pharmacy and Dental Medicine, Arad, Romania
[2] "Babes-Bolyai" University, Biological Research Institute, Cluj-Napoca, Romania
[3] University College of Nyiregyhaza, Agrar and Molecular Research Institute, Hungary
[4] "Vasile Goldis" Western University, Institute of Life Sciences, Arad, Romania

ABSTRACT

Through their rich content in polyphenolic flavonoids, the *Trigonella foenum graecum* seeds exert a protective antioxidant effect and membrane protector, certified by the works of some groups of researchers from India, the geographic area where this plant is used not only for its curative effects, but also as a flavoring food supplement. Most researches focused on the hypoglycaemic and antidiabetic action of the seeds, with only a few reports on the use as a preventive factor in the pathology of the alcoholic kidney.

Our work was conducted on an experimental model *in vivo*; in which the animals were given together with the food two different amounts of grounded seeds, against the background of alcoholic intoxication. The experimental animals were adult Wistar rats, weighing 180-200g, divided into four groups: a witness group (M), which received standard diet and water *ad libitum*; a group treated with ethanol (ER), administered in the drinking water, 10% (v/v) which received standard diet; two groups that received the same concentration of ethanol, and the food contained 5%, respectively 10% fenugreek flour (T5R and T10R). The ethanol and the flour were administered daily, for 4 weeks.

At structural level, the kidney of the animals from the ER group presented aspects of tubular vascular dystrophy, granular-vascular degeneration and vascular congestions, more evident in the cortical area of the kidneys. The electro-microscopic analysis showed at the level of the proximal convoluted tubes, vacuolation in cytoplasm, edemas at the apical pole of the nephrocytes, rarefaction of the cytoplasmic and mitochondrial matrix, the increase in number of the lysozymes and especially deperoxisomes, as well as the congestion of blood capillaries.

In the case of the groups T5R and T10R, which received Trigonella flour together with ethanol, the structural and ultra-structural changes produced by the ethylic intoxication were more attenuated, being slightly better in group T5R. The vacuolation of the cytoplasm and the number of lysozymes and peroxisomes was greatly reduced, and the aspect of the mitochondria remained in most nephrocytes similar to that of the witness animals. Most of the nuclei of the cells of the nephocytes have retained their spherical shape, being at the same time predominantly euchromatic, with little heterochromatine and evenly dispersed.

Our results plead in favor of using Trigonella seeds as a food supplement to prevent cellular deterioration and improve renal function, together with their already proven hepatoprotective effect (Rosioru et al. 2010; Pribac et al, 2010), in patients suffering from kidney and liver issues caused by excessive alcohol consumption.

Keywords: renal-protective effects – Trigonella seeds – rat kidney – structure and ultra-structure.

INTRODUCTION

Trigonella foenum graecum, popularly named Fenugreek, is an annual grassy plant from the family of the leguminous plants, whose seeds are used for a long time in the Oriental cuisine as a flavoring and condiment. Through their rich content in polyphenolic flavonoids, the *Trigonella foenum graecum* seeds exert a protective antioxidant effect and membrane protector, certified by the works of some groups of researchers from India, the geographic area where this plant is intensely used. Most researchers have mainly investigated only the anti-diabetic and hypoglycemic action of the seeds. There are no structural and ultra-structural studies on their use as a preventive factor in renal pathology in alcoholics.

Lately, plant extracts are becoming increasingly used as an alternative to drug therapy. The development of phytotherapy is also due to the fact that treatment with certain herbs and plant extracts with phytotherapeutic effect, have the advantage of obtaining them and using them at a lower price and with a low toxic potential.

Hepatobiliary and renal disorders are currently increasing, being favored by the accenting of the environmental pollution, abuse of alcohol and synthetic drugs and viral infections (Rusu et al., 2005). In pharmacies one can already find several plant extracts used for the treatment of some liver and kidney diseases, in order to neutralize the negative effects of xenobiotics. (Tămaş et al., 1995; Waterfield et al., 1993).In general, however, we mention that very few mechanisms of

action of these plant products were studied at cellular and subcelular level (Adam, 1979; Adam et al., 1979; Ciulei et al., 1993; Hrişcu et al., 1979; Vahlensieck et al., 1995). The only reference works in this area were made by Crăciun et al., 1985, 1989 a and b, 1994, 1995 a and b, 2001, 2003; Ciobanu et al., 2003; Roşioru et al.,1999, 2010; Pribac et al.,2010.

The use of plants in treating some diseases has become a tradition for a long time, "the natural pharmacy" is an important source for therapy (phytotherapy). Currently, the properties of medicinal plants are reevaluated due to the progress in chemical, clinical and pharmacological research of plants and pharmaceuticals derived from vegetal plants. Among the recognized advantages of phytotherapy we notice the lack or low toxicity, the absence of undesirable side effects and low price compared to synthetic drugs.

It is known the fact that 2% up to 10% of the consumed alcohol is eliminated almost unchanged through kidneys and lungs, while the rest of it suffers a process of oxidation at the level of liver. The ethanol molecules are small and hydrophilic, which explains its rapid absorption from the digestive tract and the alveoli, after a small quantity was already absorbed in the oral cavity (Tutunaru et al., 2007). Ethanol, also passes relatively easy through the skin and the placental barrier. Researches have shown that chronic administration of ethanol in experience/lab animals, leads to neurological dysfunctions and dysfunctions of the alimentary tract, of the liver, kidneys, heart, pancreas and causes alterations of fetus (Matsumoto and Matsumoto, 2008). Alcohol intoxication is also considered the main risk factor for inducing chronic pancreatitis in 80% of the cases. (Gullo,2005).

We underline the fact that until now, no investigation has been conducted on the effects of Fenugreek flour seeds at cellular and subcelular level upon nephocites of the alcohoolized rats, this way our study is prioritarian.

The purpose of the study was to highlight the structural and ultrastructural changes produced at the level of kidneys, especially on the proximal convoluted tubules from the renal cortex where, due to rich vascularization, there is the susceptibility of an almost direct action of the alcohol administered experimentally. We have also aimed the possible renal-protective action of the Fenugreek flour seeds, administered at the same time with the ethylic alcohol.

Our studies, particularly the ultrastructural ones, were performed on the propensity on the nephocites of the proximal convoluted tubules from the renal cortex, because of the fact that at their level occurs the reabsorption of water from the filtrate of the primary urine at a rate of 85%, being known the fact that these cells have an intense metabolic activity.

MATERIALS AND METHODS

Our experimental researches were conducted on white male Wistar rats, weighing 180-200 g. We designed an experimental model in vivo, in which the animals were simultaneous give ethanol in water and Trigonella seeds powder in food. The experience animals were grouped in four lots, each lot consisting of 10 rats, as follows:

- the witness lot (M) that received a standard diet and water ad libitum.
- The lot of animals treated only with ethanol (ER), whom were given 10% (v/v) in their drinking water, and that also consumed the standard diet.
- The lot of animals that was treated with ethanol as the above lot, and the food contained a dosage of 5% Trigonella flour seeds (T5R).
- The lot of animals that was treated with ethanol (as above) and a 10% dosage of Trigonella flour seeds in food (T5R).

The experiment lasted 31 days, after which the animals were slaughtered and we've taken kidney samples that were processed adequately in order to conduct structural and ultrastructural investigations.

We mention that all animals were starved 24 hours before being slaughtered.

For the structural studies we have used the classical histological technique and the technique of semi-fine sections.

The histological studies were made by setting, immediately after harvesting, the kidney fragments in Bouin fluid for 24 hours, followed by their subsequent processing in order to include them in paraffin.

Next we proceeded to remove the fixing solution and we immersed the organs in 70° ethylic alcohol for 24 hours; immersion in 80° ethylic alcohol for 24 hours; a bath of 95° ethylic alcohol for 24 hours, 3 consecutive baths for half an hour in benzene, and finally 2 baths in liquid paraffin at a temperature of 56° C. The organs included in paraffin were sectioned at a microtome Reichert type - Austria, to a thickness of 7µm. The sections obtained from kidney fragments were stained with hematozylin-eosin. Dyes and reagents used are Mayer hemalaun, distilled water, alcohol, hydrochloric acid, alcohol and saturated solution of lithium carbonate and 1% aqueous eosin.

The examination was conducted in an Olympus BX51 optical microscope type, the images were recorded using a CoolSnap Pro-Media Cybernetics camera.

The result of staining: nuclei stained in blue - deep purple, cytoplasm, collagen and the erythrocytes in red, the fundamental substance in red or blue, granules of secretion (where applicable), in red or blue-violet.

RESULTS AND DISCUSSIONS

Structural studies on kidney

The histological sections of kidney in group M, highlight the normal aspect of its morphology, where, at the level of the renal cortex we can observe the presence of Malpighi corpuscles, of the proximal and distal convoluted tubules, as well as of the peritubular blood vessels. The renal Malpighi corpuscle is made of a cup-shaped epithelial component – the Bowman capsule, the renal glomerulus being formed from a bundle of capillaries.The renal glomerulus is highlighted by a mass of nuclei surrounded by a void optic ring, the uriniferous space. The proximal convoluted tubules and the most part of the renal cortex, are delimited by a cubic epithelium, with large cells, having spherical nuclei located in the centre. The cells have apical "brush border" and mark the limits of a narrow lumen, the cellular limits being unclear because of the numberless lateral interdigitation. The distal convoluted tubules are delimited by a cubic epithelium, having the lumen larger than the proximal convoluted tubules (Fig.1).

The semifine highlight the normal aspect of the proximal and distal convoluted tubules (Fig. 2).

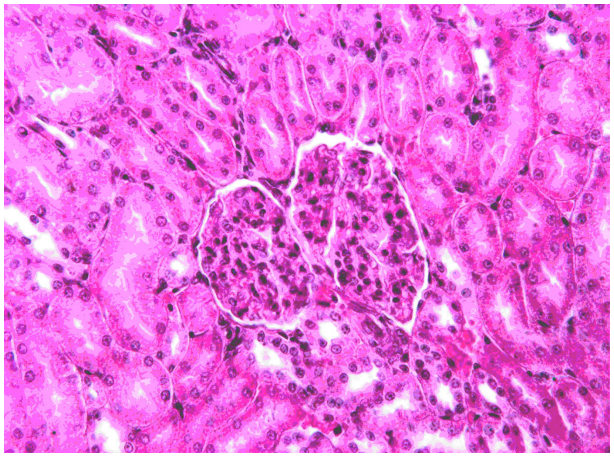

Fig. 1 Normal aspect of renal structure in M group (Ob. 40x)

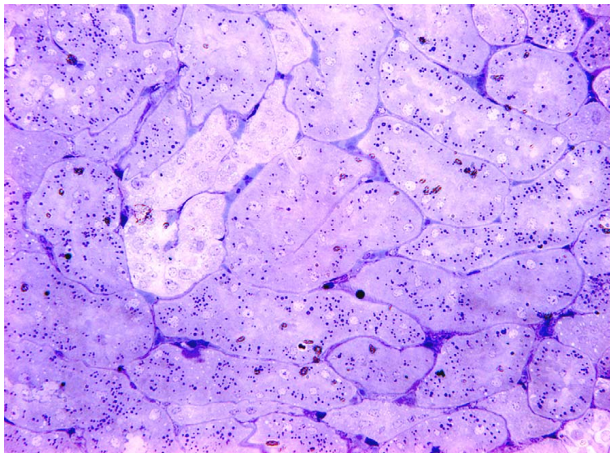

Fig. 2 Witness group (**M**), semifine section (Ob. 40x)

The treatment of animals with ethylic alcohol (group ER) determined the marked dilation of the lumen of the uriniferous tubules. The epithelium of the unriniferous tubules show aspects of vacuolar tubular dystrophy, granulo-vacuolar degeneration, the most affected components of the nephron are those located in the cortical. Thus the proximal tube and its distal segment are the most affected segments, the lesions were present in a variable number of nephrons. Some uriniferous tubes have epithelial desquamation, without affecting the integrity of the tubular basal membrane, which further promotes the process of regeneration and recovery. Part of the proximal and distal convoluted tubules present epithelial cells grown in volume, in the cytoplasm we can see small granules, of different sizes, which are spread homogeneous. In other epithelial cells of the proximal and distal convoluted tubules we can observe the appearance of small vacuolar formations, disseminated in the cytoplasm (Fig. 3). These aspects suggest a granular and vacuolar dystrophy, expression of some hydropic changes or intracytoplasmic glycogen deposits. At the level of the glomerulus there were detected some lesions characterized by the thickening of the glomerular capillary loops, which are associated with dilatation of the intraglomerular distances (Fig. 4). Alcohol, which has vasodilator effect, determined the congestion of the lumen of the blood vessels, in some areas we can also observe the clutter of red blood cells, (Fig. 5). Noteworthy is the presence at the level of numerous proximal convoluted tubes and of numerous dense granules, most of them being peroxisomal and partially lysosomal (Fig. 6). It is known as a fact that the peroxisoms play an important role in detoxification processes, a function which is facilitated by the very big permeability for ions and small molecules of the peroxisomal membrane. The catalase of the peroxisoms oxidizes alcohol. It is considered that half of the alcohol consumed by drinkers is oxidized to acetaldehyde at the level of peroxisoms.

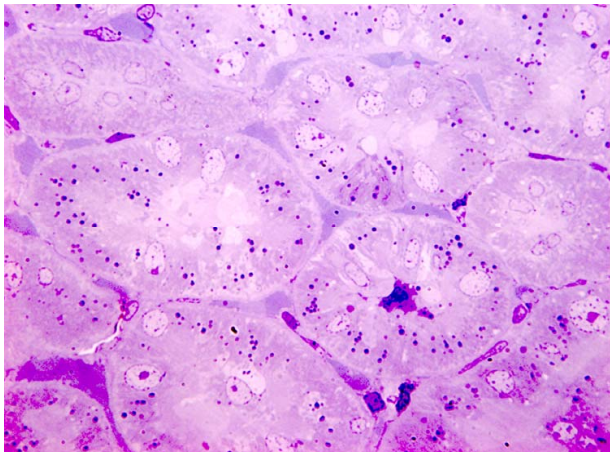

Fig. 3 The group treated with alcohol **(ER)**, semifine section (Ob. 100x)

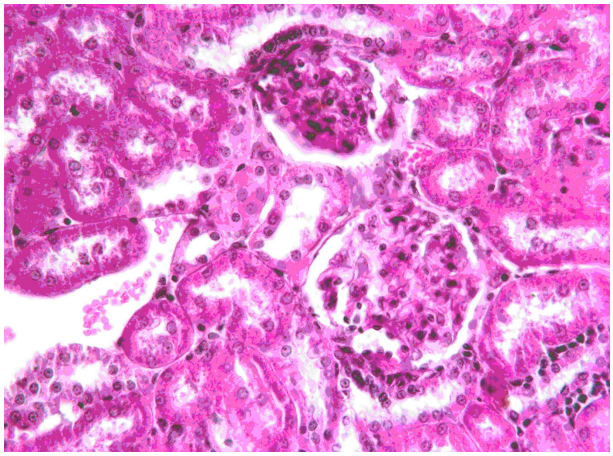

Fig. 4 The group treated with alcohol **ER** – Highlighted tubular dilatations, garnular and vacuolar degeneration associated with peeling of the epitheliums of the uriniferous tubes. In the lumen of the uriniferous tubes we observed the presence of red cells, (Ob. 40x)

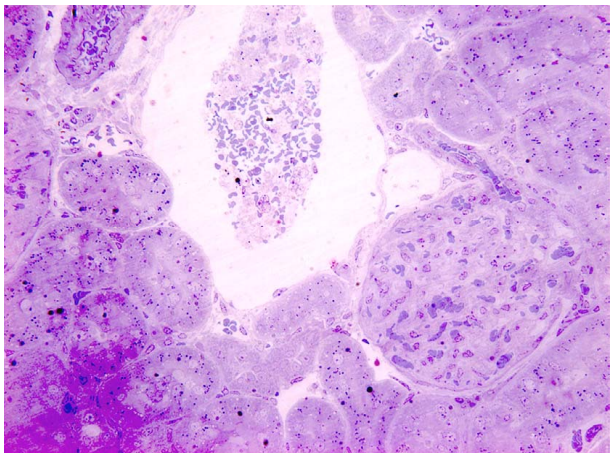

Fig. 5 The group treated with alcohol **(ER)** (Ob. 40x)

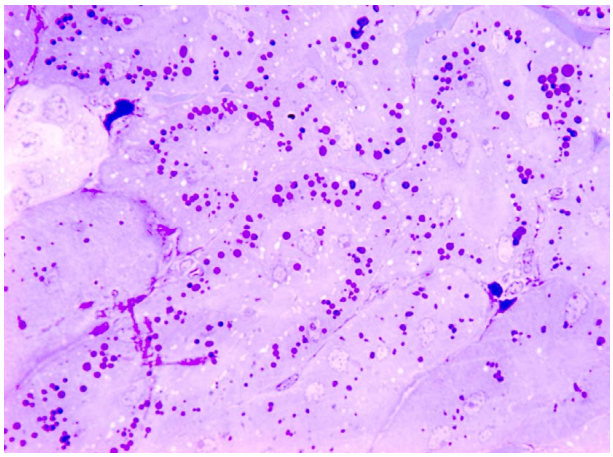

Fig. 6 The group treated with alcohol **(ER)**, semifine section (Ob. 100x)

In the group treated with a dose of 5% of Fenugreek flour (T5R group) a significant reduction of glomerular tubular and dystrophic phenomena was revealed, compared with the renal morphology changes occurring in succession with alcohol treatment of animals.

Thus, the histological examination of the kidney revealed moderate changes in the structure of the renal parenchyma, both at the level of the cortex and of the bone marrow. At the level of the renal glomeruli small lesions were detected, characterized by a discrete thickening of the glomerular capillary loops (Fig.7 and 8).

In some uriniferous tubes aspects of moderate epithelial desquamation were highlighted, as well as dilatations of the lumen, especially in their proximal portions. The described aspects are more pronounced at the level of the proximal tubule and of the Henle loop. Both in the cortical as well as in the renal medullary, there were found dilatations of the blood vessals, but they have no morphopathological character (Fig. 9).

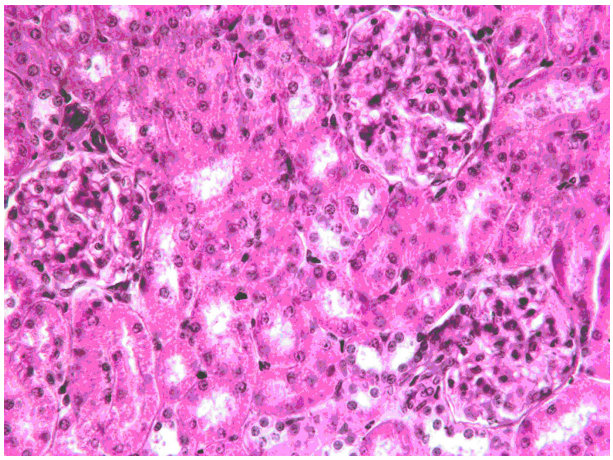

Fig. 7 – Discrete thickening of the glomerular capillary loops; moderate epithelial vacuolar degeneration; dilatations of the lumen of the uriniferous tubes in groupT5R, (Ob. 40x).

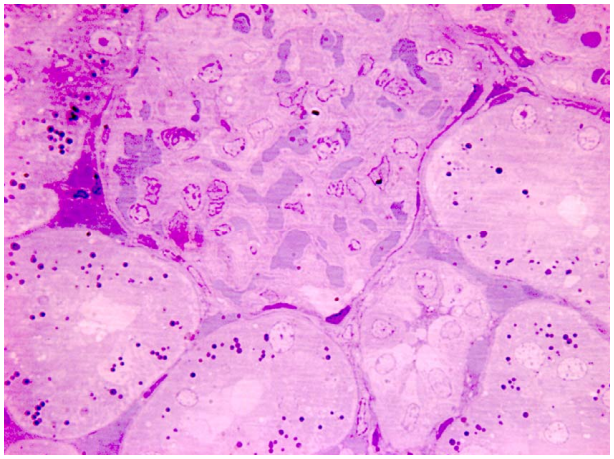

Fig. 8 Group **(T5R),** semifine section (Ob. 100x)

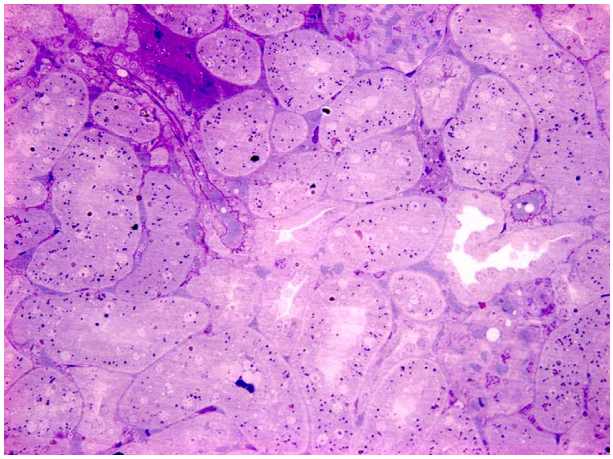

Fig. 9 Group **T5R,** semifine section (Ob. 40x)

The administration of 10% Fenugreek flour (T10R), has had a repairing effect, but a little bit more moderate compared to the previous group which was treated with 5% Fenugreek flour. We have observed aspects of epithelial degeneration, as well as dilatations of the tubular lumen, and in several fragments, both of the convoluted tubules as well as of the listal ones, the epitheliums are peeled (Fig. 10 and 11). The most affected segments of the nephron are also those located in the cortical. At the level of some glomeruli there were found small lesions, manifested by intercapilar or mesangial expansion space associated with a thickening of the glomerular capillary loops (Fig. 12). Some of the blood vessels are still dilated, as a vasodilator effect of the alcohol and show congestion with red blood cells (Fig. 13).

The results of the structural studies regarding the administration of Trigonella seeds powder containing 5% respectively 10% Fenugreek flour on kidney morphology, suggest the trens of a reparatory effect of these herbal preparations in the structural and functional recovery of the kidney animals intoxicated with ethylic alcohol.

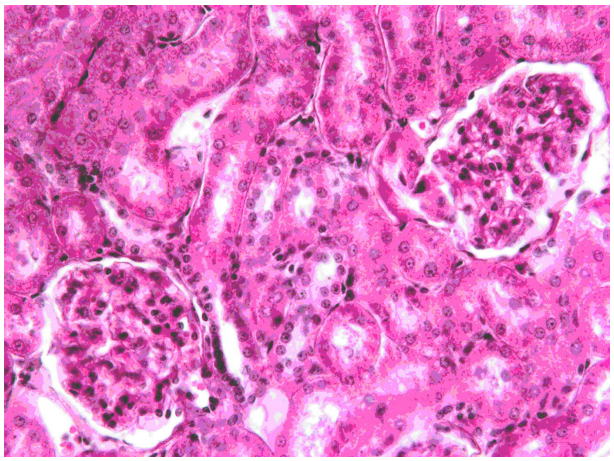

Fig. 10 – Group T10R – Dilatations of the mesangial glomerular space, moderate granular and vacuolar epithlial distrophy, more highlighted dilatations of the lumen of the uniferous tubes,(Ob. 40x).

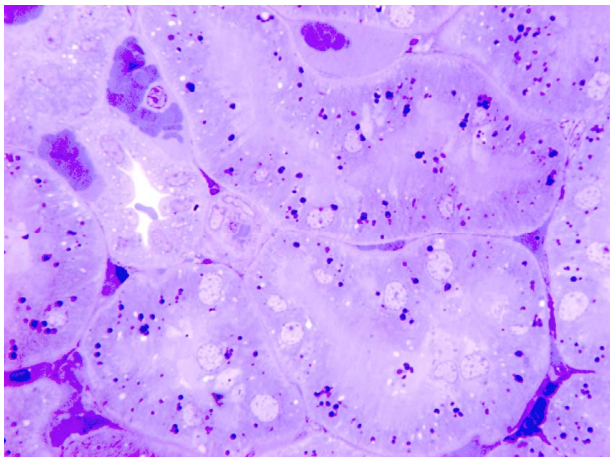

Fig. 11 Group **T10R**, semifine section (Ob. 100x)

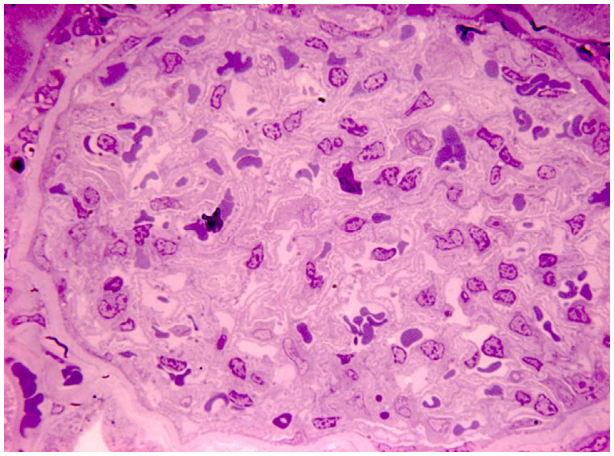

Fig. 12 Group **T10R**, semifine section (Ob. 100x)

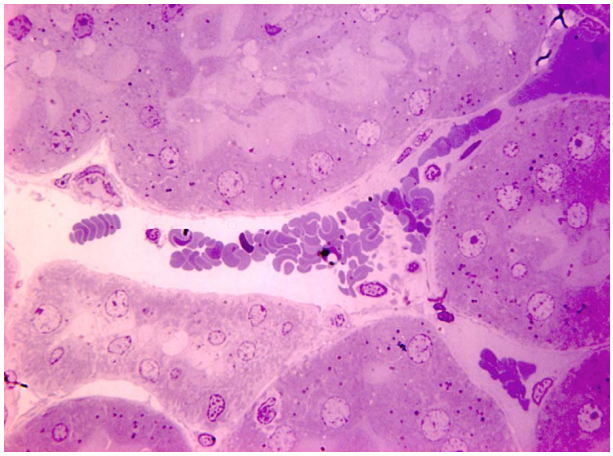

Fig. 13 Group **T10R**, semifine section (Ob. 100x)

CONCLUSIONS

The experimental administration of ethylic alcohol in rats induces various structural and ultrastructural changes at the level of the kidney, more pronounced at the level of the renal cortex and especially at the level of the proximal nephrocytes of the convoluted tubules.

The concurrent administration of the Fenugreek flour produced an improvement of the negative effects caused by alcohol, proving to lead an activity of protection of the renal structure and function.

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